

**Background:** The mechanisms regulating cross-bridges (XBs) cycling during stretch and shortening are controversial. We hypothesize that XB strong to weak transition (weakening) rate increases during shortening and decreases during lengthening in an identical velocity dependent manner. Our hypothesis reproduces the muscle basic properties as the force-velocity relationship and regulation of energy consumption. The study investigates this unifying hypothesis during lengthening and shortening. **Methods:** Trabeculae were isolated from rat right ventricles ( $n=9$ ). Sarcomere length was measured by laser diffraction. The number of strong XB ( $N_{XB}$ ) was evaluated by measuring the dynamic stiffness. Stretches ( $n=42$ ) and releases ( $n=48$ ) at different velocities and instants were imposed on sarcomere isometric contractions. **Results:** Faster stretches yielded larger forces. An overt identical linear correlation between force and  $N_{XB}$  development was obtained for any stretch velocity ( $0.2\text{--}1.7\mu\text{m/s}$ ), implying that the force increased due to the increase in  $N_{XB}$ , whereas the unitary force per XB ( $F_{XB}$ ) was constant. The stiffness development rate linearly depended on the lengthening velocity with a proportion coefficient of  $6.9 \pm 0.46$ . Shortening yielded both a decrease in  $N_{XB}$  and  $F_{XB}$ . Interestingly, the stiffness decline rate depended linearly on the shortening velocity ( $0.6\text{--}2.7\mu\text{m/s}$ ) with similar proportion coefficient of  $6.08 \pm 2.45$ . When identical perturbation (lengthening or shortening) was imposed at different instants during the twitches, similar rate of change in the stiffness and force development were observed. Thus, the phenomena are not dominated by  $N_{XB}$  but relate to an inherent property of the single strong XB. **Conclusions:** The independence of XB weakening rate on the perturbation onset time and the identical dependence on the velocity during shortening and lengthening strongly support the hypothesis that XB dynamics is dominated by a single velocity dependent kinetic.

## Cytoskeletal Protein Dynamics

### 2865-Pos

#### Escherichia Coli Single FtsZ Dynamic Rings: Growing, Cyclization, Opening, Reannealing and Depolymerization

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The prokaryotic homologue of tubulin FtsZ plays a major role in cell division. *In vivo* it is localized at the center of the bacteria forming a dynamic ring that constricts during division. *In vitro* it binds and hydrolyzes GTP, and polymerises in a GTP-dependent manner. The role played by the hydrolysis in filament stability is not well understood.

In this work we have studied isolated FtsZ protofilaments by AFM obtaining information about the polymerization, steady state and depolymerization process in real-time at the single-molecule level. Previous experiments with Atomic Force Microscopy (AFM), which provide both high structural resolution and dynamics information under buffer solution, have reported complex dynamic behaviour for FtsZ filament network deposited onto a mica surface<sup>[1]</sup>. A recent model considering a labile longitudinal monomer-monomer bond with a preferential curvature and flexibility in addition to lateral attraction between monomers is enough to account for this rich dynamic behaviour<sup>[2]</sup>.

In order to correlate GTPase activity with the labile nature of longitudinal bond between FtsZ monomers we used slow hydrolyzable GTP analogues. We found a strong decrease on depolymerization velocity when GTPase activity is diminished allowing us to reveal cyclization, fragmentation, reannealing and release of monomers from either end or from both simultaneously as the essential features of a single FtsZ filament dynamics.

[1] J. Mingorance, M. Tadros, M. Vicente, JM. González, G. Rivas and M. Vélez J. Biol.Chem. , 280, 20909-20914, (2005)

[2] A. Paez, P. Mateos-Gil, M. Vélez and P. Tarazona, Soft Matter, 5, 2625-2637 (2009)

### 2866-Pos

#### The Mechanism of E-Ring Formation During Min Oscillations

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In the bacterium *Escherichia coli*, the Min proteins oscillate between the cell poles to select the cell center as division site. This dynamic pattern has been proposed to arise by self-organization of these proteins, and several models have suggested a reaction-diffusion type mechanism. Recently, we have found that the proteins

MinE and MinE are able to spontaneously form planar surface waves on a flat membrane *in vitro*. One particular feature of the patterns *in vivo* and *in vitro* is the so-called MinE ring, which defines the directionality of the traveling wave. How this accumulation of MinE at the trailing edge of the wave arises and its functional role during Min oscillation is not yet fully understood. We have applied FRET, TIRF and single molecule imaging techniques to study the behavior and interdependence of the three Min proteins in more detail. This helped us to propose a novel mechanism of Min protein wave propagation and E-ring formation.

### 2867-Pos

#### Kinetic Analysis of Actin Dendritic Nucleation from a Physicist's Standpoint

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Arp2/3-mediated polymerization of actin is an important mechanism by which cells dynamically change their shapes. Recent advances in deciphering a pathway of dendritic nucleation lay the groundwork for kinetic analyses that would estimate rate constants of the reaction steps involved in the process. Using kinetic models with various assumptions about a pathway's rate-limiting step, we have analyzed published quantitative data obtained from pyrene-labeled actin assays. All the available data are fitted well by two different models: one assumes that the nucleation is limited by the binding of the Arp2/3 complex to a mother filament whereas in the other, the rate-limiting step is the subsequent activation of the bound complex. To distinguish between these possibilities conclusively, we propose experiments with varying initial concentrations of actin monomers. The experiments need to be performed in nonequilibrium conditions, in which spontaneous formation of aster-like actin structures have been recently observed. For these conditions, the two models exhibit qualitatively different dependences of fluorescence intensity on the initial concentrations of G-actin. The work is supported by National Institutes of Health through grants 1U54 -RR022232, P41-RR13186, and 1U54-GM64346-01.

### 2868-Pos

#### Simulation Reveals Fundamental Behavior of the Actin Filament and Arp2/3 Branch Junction

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Molecular modeling and simulation now offer unprecedented opportunity to study biological events at the atomic level. Unfortunately, broad application of these approaches is elusive due to the difficulty of simulating biologically relevant length and time scales. I will use our recent investigation into key processes within the actin cytoskeleton to highlight progress we have made on these challenges. The self-assembly of actin into a branched network of filaments is a vital process within the cytoskeleton of eukaryotic cells. This is chiefly regulated by the controlled hydrolysis of actin's bound ATP nucleotide. Although it is well established that ATP hydrolysis induces a change in actin's structure and dynamics, the exact mechanism by which this occurs is still unknown. We have used a novel computational approach to simulate various hydrolysis-induced structural transformations that have been suggested in the literature. Based on these results we are able to suggest a mechanism by which ATP hydrolysis regulates actin's structure and dynamics.

Equally important to the dynamics of the cytoskeleton is the process by which actin filaments are depolymerized in a controlled fashion. Recent experimental studies have yielded a number of hypotheses regarding the process by which actin depolymerization factor cofilin severs and depolymerizes filaments. We will present molecular and coarse-grained analyses of cofilin-bound actin filaments, and provide a molecular view of this process.

Finally, we have performed simulations of the actin branch junction - a key structural building block in the cytoskeleton. Our simulations reveal how small features of the actin protein give rise to the underlying dynamics of the entire branch junction. Strategies for coarse-graining supramolecular structures will be discussed in context of the branch junction.

### 2869-Pos

#### Toward De Novo Recapitulation of Cytoskeleton Dynamics with DNA Nanotubes

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The polymerization and depolymerization of cytoskeleton can transduce chemical energy into mechanical energy that drives cellular motility, such as chromosome segregation to cell protrusion. De novo recapitulation of the cytoskeletal phenomena with synthetic material would test our understanding of the design principles of polymerization motors. In the DNA nanotechnology tool box, DNA nanotubes are arguably the closest counterparts of cytoskeleton